FINAL PROJECT REPORT
WTFRC Project Number: AP-09-901AB

Project Title: A sensitive indicator of Honeycrisp fruit N status for maximal quality

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Total Project Funding: **Year 1:** $34,595 **Year 2:** $35,391

Budget 1
Organization Name: Cornell University

Item	Year 1 (2009)	Year 2 (2010)	
Salaries	11,500	11,960	
Benefits	6,095	5,856	
Supplies	6,100	6,675	
Travel	900	900	
Total	24,595	25,391	

Footnotes:

Salaries budgeted are for a 4-month postdoc working on this project at $34,500 per year in the first year and at $35,880 in the second year. The fringe benefit rate for the second year is 48.96%.

Supplies include cost of leaf and fruit nitrogen analysis, analytical columns, guard columns, standards, solvents, vials and service for the HPLC separation and quantification of amino acids.

Travel expense is budgeted for one trip per year to the experimental site in WA to set up the trial and collect samples.

Budget 2
Organization Name: Cornell University

Item	Year 1 (2009)	Year 2 (2010)	
Fruit loss compensation*	10,000	10,000	
Total	10,000	10,000	

Footnotes: * This was a contingency plan for the worst case scenario where the high N treatments (120lbs and foliar N treatments) might make some of the fruit unmarketable so that the co-operative grower gets compensated for the loss: 20 bins of unmarketable fruit x $500/bin = $10,000. We only used a small portion of the budget in 2009 and we did not use any in 2010.

Objectives

The objectives of this project are 1) to determine how asparagine and other free amino acids in Honeycrisp fruit respond to N supply and relate the levels of free amino acids to fruit N status and fruit quality and 2) establish the optimal range of fruit N status expressed as asparagine level, with the goal of developing a sensitive indicator of Honeycrisp fruit N status for maximal quality.

Significant Findings

- Over the range of N fertilization rate 0 to 120 lbs per acre, N fertilization had only limited effect on fruit yield, fruit size, quality and physiological disorders during the first two years of the trial. Although trees in the highest N treatment tended to have higher yield and bigger fruit, N fertilization beyond 30 lbs/acre didn't bring any significant benefit.

- Both leaf N and fruit N increased only slightly in response to increasing rate of N fertilization. However, fruit asparagine concentration increased significantly when N fertilization rate increased from 0 to 30 lbs N per acre. Any further increase in N fertilization rate didn't result in significant increase in fruit asparagine concentration.

- Asparagine accounted for about half of the total free amino acids in Honeycrisp fruit. As a result of the increase in asparagine concentration, the concentration of total free amino acids increased in the same way as asparagine in response to N fertilization.

- Both fruit asparagine concentration and total free amino acid concentration are more sensitive than leaf N or fruit N in response to N fertilization.

Methods

Two field trials were set up in commercial Honeycrisp orchards in WA in 2009, one at Brewster Flats and the other at Naches Heights. The trees at Brewster Flats were 6-yr-old trees on M.26 at 8 x 14 foot spacing (389 trees/acre). The trees at Naches Heights were 5-yr-old over-grafts on Delicious/MM106 at 6 x 16 foot spacing (454 trees/acre). At both sites, trees were fertilized with 0, 30, 60, 90, or 120 lbs actual N per acre in 2009, whereas in 2010 only the trial at Brewster Flats was continued. Each treatment was replicated 5 or 6 times, with 5 to 6 trees per replicate, in a randomized complete block design. For each nitrogen treatment, urea fertilizer was applied at tight cluster and 3 weeks later in an equal split. The fertilizer was spread under the tree canopy and was watered into the soil within a few days of application. The cropload of the trees at Brewster site was 5 to 7 fruit per cm^2 trunk cross-sectional area (TCA) and that at Naches site was 9 to 10 fruit per cm^2 TCA, after chemical thinning followed by hand-thinning.

In addition, a field trial was set up at Hill Top orchards in 2010 to use foliar urea applications during fruit development as a method to generate a range of fruit N status. Briefly, 7-yr-old Honeycrisp/M26 trees at 3 x 15 foot spacing (968 trees/acre) received 0, 1, 3, 5, or 7 foliar sprays of 5 lbs urea/100 gal water at weekly intervals centered around 6 weeks before expected harvest in 2010. Each treatment was replicated 5 times in a randomized complete block design with 4 trees per replicate. The cropload of the experimental trees was adjusted to 5 to 7 fruit per cm^2 trunk cross-sectional area (TCA) after chemical thinning followed by hand-thinning.

The effects of N treatments on leaf and fruit N status, levels of free amino acids, yield and fruit quality were monitored. Leaf samples were taken in mid-August for nitrogen analysis. Fruit samples were taken at harvest for analysis of N and individual free amino acids. Fruit yield and size were measured at harvest and one bushel of fruit per replicate was stored in a regular cold room. Fruit quality (color, firmness, soluble solids, and occurrence of bitterpit and other disorders) were assessed at fruit harvest and after 4 months of cold storage. Asparagine and all other free amino acids were

separated and quantified with an Agilent 1100 high performance liquid chromatography using the AccQ-Tag method (Waters Corporation).

Results and Discussions

1. Effects of N fertilization on fruit yield, fruit size and quality

At Brewster site, all the trees had relatively low cropload in 2009. Fruit number and fruit yield per tree were higher in the highest N treatment (120 lbs/acre) than in the control (Table 1). No significant difference in fruit size was detected between any N treatments and the control, but the trees fertilized with nitrogen tended to have slightly bigger fruit. N fertilization did not significantly affect fruit firmness, soluble solids, acids, or bitterpit incidence. Percentage of clean fruit (free of russet) was significantly higher in the 120 lbs N treatment than in the control. In 2010, tree cropload was medium. Compared with the control, fruit size was slightly larger, and correspondingly fruit firmness was slightly lower in the N treatments (Table 2). As N rate increased, percentage of fruit with greasiness increased. Sunburn incidence was significantly higher at 120 lbs N rate than the control. N fertilization did not significantly affect fruit soluble solids, acids, color, bitterpit incidence or russet incidence.

At the Naches site, all the trees had relatively high cropload in 2009. N fertilization did not significantly affect fruit number or fruit yield per tree (Table 3). This may be partly due to the fact that the grower had put on about 30 lbs of nitrogen on all the trees before the trial was set up. Fruit size was the largest in the highest N treatment (120 lbs/acre), but for some reason, trees in the 30 lbs N /acre treatment produced the smallest fruit. Fruit firmness was slightly affected by N fertilization. No difference in fruit soluble solids or acids was detected between any N treatments and the control. Bitterpit was highest in the control, which we don't have a clear explanation for. Perhaps the trees were relatively vigorous and fruit size was relatively big. Sunburn incidence at this site was higher than that at Brewster site in 2009, and N fertilization slightly affected sunburn incidence. Similar to that found at Brewster site, percent of clean fruit was significantly higher in the highest N treatment (120 lbs N/acre) than in the control.

No difference in fruit yield, fruit size, firmness, soluble solids, acids, or any disorder was detected between foliar N-treated and the control (Table 4).

2. Effects of N treatments on leaf N, fruit N, fruit asparagine and total free amino acids

At Brewster site, both leaf N and fruit N increased only slightly from 1.9% to 2.2% and from 0.24% to 0.31% respectively in 2009, and from 2.1% to 2.4% and from 0.20% to 0.32% respectively in 2010, as N fertilization rate increased from 0 to 120 lbs/acre (Fig 1). However, both fruit asparagine concentration and total free amino acid concentration increased significantly. In 2009, fruit asparagine concentration and total free amino acid concentration increased from 4.4 to 7.2 mg/g and from 10.4 to 13.9 mg/g, respectively in 2009 when N fertilization rate increased from 0 to 30 lbs/acre. Any further increase in N fertilization rate (beyond 30 lbs/acre) did not result in any significant increase in either fruit asparagine concentration or total free amino acid concentration. In 2010, the increase in both asparagine concentration and total free amino acid concentration in response to N fertilization was larger at the same N rate (Fig. 1).

At Naches site, leaf N was higher than at Brewster site, but it increased only slightly at the two highest N fertilization rates (Fig 1). Surprisingly, fruit N levels were slightly lower than those at Brewster site, but it increased from 0.30% to 0.37% as N fertilization rate increased from 0 to 120 lbs/acre. Both fruit asparagine concentration and total free amino acid concentration showed the same trend in response to N fertilization: they increased significantly (from 6.8 to 9.3 mg/g and from 13.2 to 16.8 mg/g) when N fertilization rate increased from 0 to 30 lbs/acre, but no additional significant increase was detected with further increase in N fertilization rate.

At the Hill Top site, fruit N did not respond significantly to foliar urea application (Fig. 2). Both fruit asparagine concentration and total free amino concentration showed an increase when

foliar urea was applied one time, but no further increase was observed when the number of foliar urea applications was increased beyond one application.

Compared with leaf N and fruit N, fruit asparagine concentration and total free amino acid concentration were more sensitive to N supply, and therefore should be better indicators of fruit N status. Since both fruit asparagine concentration and total free amino acid concentration responded more to the same soil N fertilization during the second year (2010) than during the first year (2009), and we only have one-year data on foliar urea application, both trials need to be repeated for another season in order to draw firm conclusions.

Table 1. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Brewster site, 2009)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Bitter pit (%)*	*Sunburn (%)*	*Russet % clean*
0	11.1b	61b	180	18.1ab	12.3	0.529	1	7ab	39b
30	18.1 ab	95 ab	187	18.3 a	11.8	0.485	1	8 ab	39 b
60	18.5 ab	98 ab	186	17.9 b	12.7	0.474	1	9 a	43 ab
90	14.7 ab	73 ab	191	18.2 ab	12.3	0.520	2	5 ab	49 ab
120	22.5 a	119 a	192	18.0 ab	12.4	0.532	1	1 b	68 a

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 2. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Brewster site, 2010)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Grease (%)*	*Sunburn (%)*	*Russet % clean*
0	23.0	89	210 b	16.0 a	12.9	0.47	5 c	3 bc	0
30	25.7	97	228 ab	15.5 ab	13.4	0.5	21 b	1 c	0
60	25.2	95	231 a	15.6 ab	13.1	0.50	15 bc	9 ab	0
90	23.0	86	229 ab	15.5 ab	13.4	0.52	25 b	5 bc	2
120	29.0	109	223 ab	15.5 b	13.1	0.49	47 a	15 a	4

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 3. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Naches site, 2009)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Bitter pit (%)*	*Sunburn (%)*	*Russet % clean*
0	43.0	216	217ab	16.9bc	12.1	0.507	11a	16ab	57b
30	43.1	237	187 b	17.2 ab	12.5	0.499	4 b	21 a	72 ab
60	42.6	229	201 ab	17.5 a	12.6	0.537	3 b	20 a	68 b
90	49.4	274	191 ab	16.8 c	12.3	0.497	2 b	10 b	59 b
120	48.2	231	222 a	16.7 c	12.6	0.540	3 b	13 ab	85 a

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 4. Effects of foliar urea sprays on Honeycrisp yield, fruit size and quality (Hilltop site, 2010)

Sprays	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Grease (%)*	*Bitterpit (%)*	*Russet % clean*
0	36.8	127	285	13.3	12.1	0.45	0	7	N/A
1	36.8	123	296	13.5	12.3	0.47	0	8	N/A
3	38.2	128	305	13.3	11.8	0.47	0	15	N/A
5	43.9	151	305	13.3	12.3	0.44	0	10	N/A
7	41.8	142	282	13.4	11.8	0.41	0	2	N/A

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

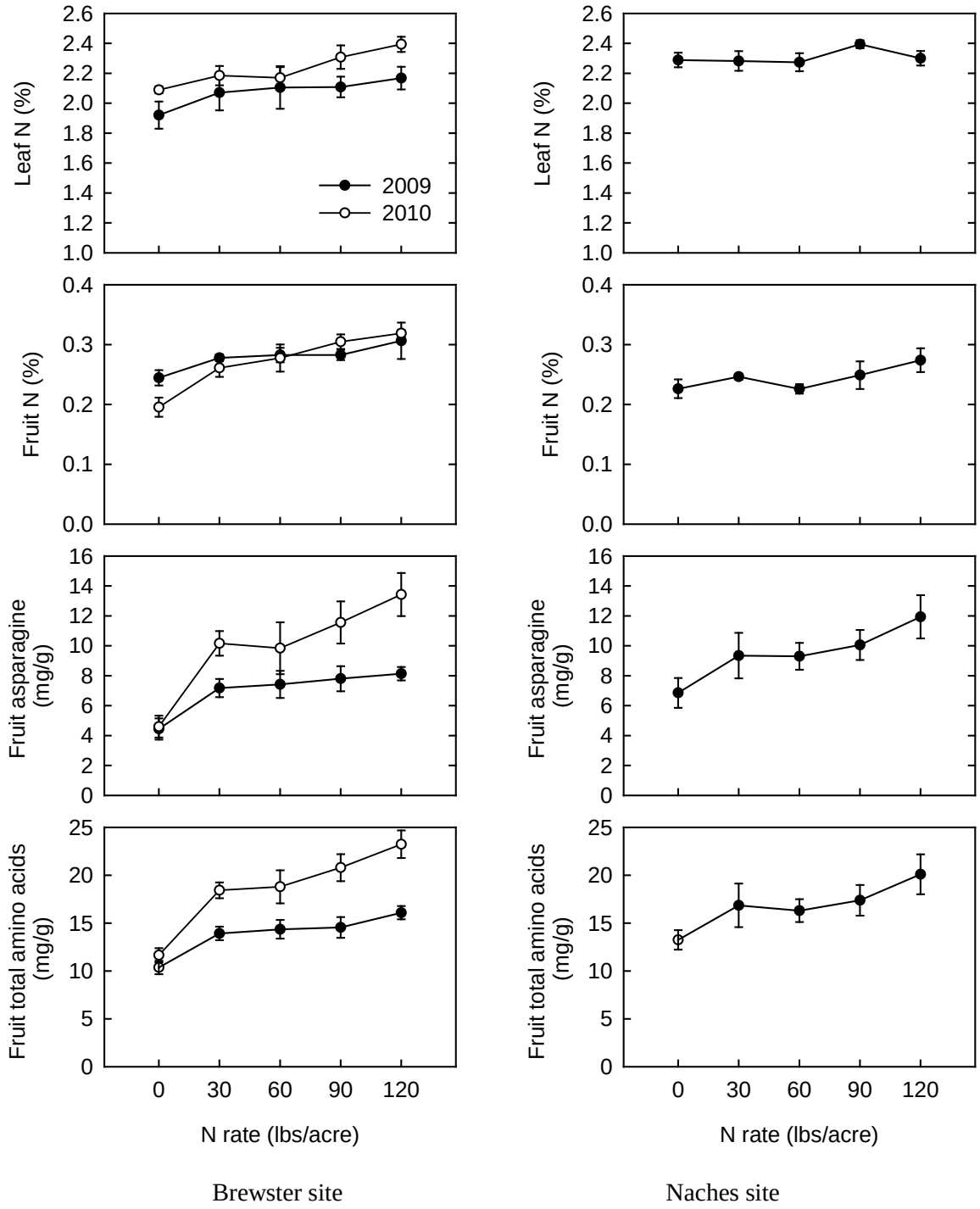

Fig 1. Nitrogen content of leaf samples taken in mid-August, and concentrations of nitrogen, asparagine and total free amino acids in Honeycrisp fruit at harvest in response to nitrogen fertilization at both Brewster site and Naches site.

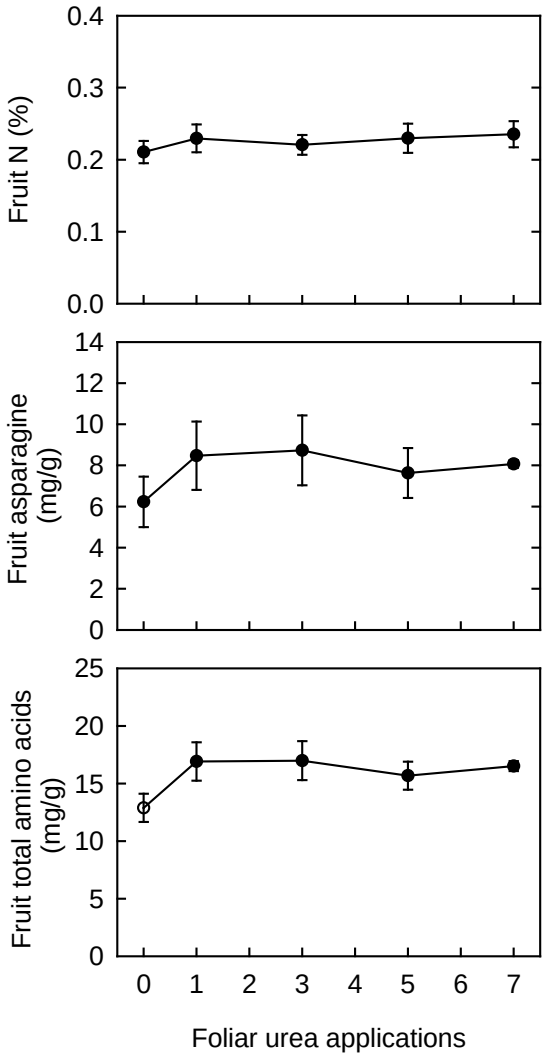

Fig. 2. Concentrations of nitrogen, asparagine and total free amino acids in Honeycrisp fruit at harvest in response to foliar urea applications

Executive Summary

The objectives of this project are 1) to determine how asparagine and other free amino acids in Honeycrisp fruit respond to N supply and relate the levels of free amino acids to fruit N status and fruit quality and 2) establish the optimal range of fruit N status expressed as asparagine level, with the goal of developing a sensitive indicator of Honeycrisp fruit N status for maximal quality. Over the last two years, we have used both soil application of nitrogen and foliar urea sprays to alter tree and fruit N status. The effects of N supply on fruit yield and quality and the concentrations of asparagine and total free amino acids in fruit were evaluated.

Over the range of N fertilization rate 0 to 120 lbs per acre, N fertilization had only limited effect on fruit yield, fruit size, quality and physiological disorders during the first two years of the trial. Although trees in the highest N treatment tended to have higher yield and bigger fruit, N fertilization beyond 30 lbs/acre didn't bring any significant benefit. Both leaf N and fruit N increased only slightly in response to increasing rate of N fertilization. However, fruit asparagine concentration increased significantly when N fertilization rate increased from 0 to 30 lbs N per acre. Any further increase in N fertilization rate didn't result in significant increase in fruit asparagine concentration. Asparagine accounted for about half of the total free amino acids in Honeycrisp fruit. As a result of the increase in asparagine concentration, the concentration of total free amino acids increased in the same way as asparagine in response to N fertilization. No significant effect on fruit yield, quality, or fruit N content was found in foliar urea treatments. However, both fruit asparagine concentration and total free amino concentration showed an increase when foliar urea was applied once, but no further increase was observed when the number of foliar urea sprays was increased further.

Compared with leaf N and fruit N, fruit asparagine concentration and total free amino acid concentration were more sensitive to N supply, and therefore should be better indicators of fruit N status. Since both fruit asparagine concentration and total free amino acid concentration responded more to the same soil N fertilization during the second year (2010) than during the first year (2009), and we only have one-year data on foliar urea application, both trials need to be repeated for another season in order to draw firm conclusions.